ON THE MECHANISM OF RESISTANCE TO ARYLOXYPHENOXYPROPIONATE AND CYCLOHEXANEDIONE GRAMINICIDES IN ANNUAL RYEGRASS (*LOLIUM RIGIDUM*)

J.A.M. Holtum, J.M. Matthews, D.J. Liljegren, R.E. Häusler and S.B. Powles
Waite Agricultural Research Institute, University of Adelaide,
P.M.B. 1 Glen Osmond, South Australia 5064.

INTRODUCTION

Over 50 populations of *L. rigidum*, collected from all Australian mainland broadacre cropping regions, have been documented to be resistant to herbicides with different modes of action. Herbicides against which there is resistance include the aryloxyphenoxypropionates, cyclohexanediones, sulfonylureas, imidazolinones and dinitroanilines (2, 5, 6, 8). Many more populations are known to be resistant but have not been characterised. A common feature of all populations documented to date is that they exhibit resistance to diclofop-methyl (Figure 1).

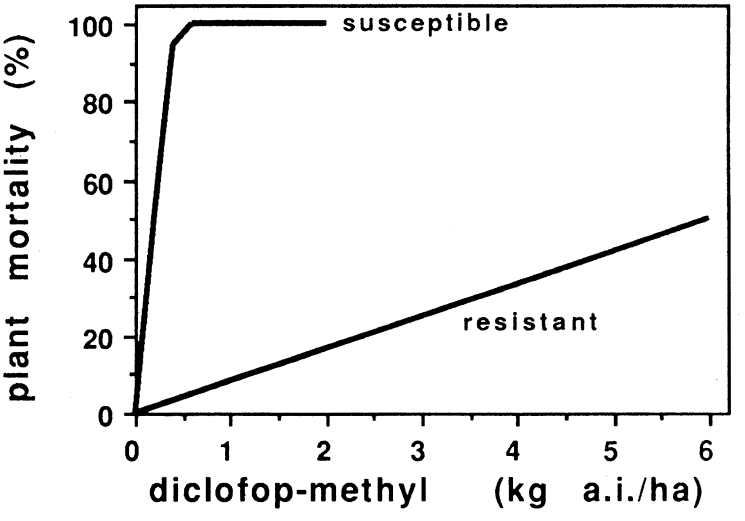

Figure 1. Responses of a susceptible and a resistant population of *L. rigidum* to diclofop-methyl. Typical field rate is 365 g. a.i./ha.

The extent of resistance to other aryloxyphenoxypropionate and to cyclohexanedione graminicides is population-dependent (cf. 6). We are attempting to determine the biochemical mechanisms responsible for resistance.

RESULTS AND DISCUSSION

Acetyl CoA carboxylase (ACCase) The aryloxyphenoxypropionate and cyclohexanedione graminicides, which include diclofop-methyl, fluazifop-butyl, haloxyfop-methyl, quizalafop-ethyl, fenoxaprop, sethoxydim, tralkoxydim, alloxydim, clethodim and cycloxydim, inhibit the enzyme ACCase. The enzymes from most grasses are more sensitive to inhibition by these compounds than are the enzymes from dicotyledonous plants (1). Resistance to diclofop-methyl in *L. multiflorum* and resistance to sethoxydim in maize cell cultures is probably due to the development of tolerant forms of ACCase (3). As *L. rigidum* exhibits resistance to all of the above-mentioned graminicides we suspected that the mechanism of resistance in *L. rigidum* may be the product of an altered sensitivity of ACCase. This possibility was examined and, as will be demonstrated, discarded. ACCase from susceptible population SRS2 and resistant population SR31 was equally inhibited by a range of graminicides (Table I). The concentrations required to inhibit ryegrass enzyme activities by 50 % were, in the main, similar to those required to inhibit the enzyme from wheat but less than those required to inhibit the enzyme from peas. The tolerance of wheat to diclofop does not appear to be due to the low sensitivity of wheat ACCase to diclofop.

Table I. Concentrations of graminicides that inhibit ACCase activity by 50 %.

compound	susceptible ryegrass	resistant ryegrass	wheat	pea
		[μM]		
diclofop acid	0.2 ±0.01	0.3 ±0.07	0.3	3.0
haloxyfop acid	0.4 ±0.07	0.7 ±0.1	1.1	4.3
fluazifop acid	0.6	1.8	2.0	>10
sethoxydim	2.7 ±0.6	2.5 ±0.22	1.0	>10
tralkoxydim	0.3 ±0.09	0.4 ±0.11	4.2	>10

Subsequent experiments demonstrated that the amounts and kinetic characteristics of ACCase from susceptible and resistant ryegrass were similar both in plants that had been sprayed with diclofop-methyl and in plants that had not been sprayed (7). Clearly resistance to diclofop acid, fluazifop acid, haloxyfop acid, sethoxydim and tralkoxydim in the population of *L. rigidum* studied is not due to a reduction in the sensitivity of ACCase to these compounds nor to the amount of the enzyme present.

The fate of diclofop-methyl within *L. rigidum* In order to test whether resistance was due to (i) differences in the rates of entry of diclofop-methyl into ryegrass or (ii) biotype-specific differences in the rates or pathways of breakdown of diclofop-methyl, radiolabelled herbicide was fed to both biotypes and the uptake and fate of radioactivity was monitored. Foliar-applied ^{14}C-U-diclofop-methyl is taken up at similar rates by two-leaved susceptible and resistant ryegrass plants. The rates of breakdown of diclofop-methyl were also similar (Figure 2A).

Diclofop acid, a metabolite with greater herbicidal activity than diclofop-methyl, accumulated in the susceptible plants at a faster rate than in resistant plants (Figure 2B). The pool size in the susceptible plants was greater than that in the resistant plants throughout the duration of the experiment. In both biotypes the extractable activities of an esterase capable of transforming diclofop-methyl to diclofop acid were similar. Resistant plants accumulated herbicidally-inactive conjugated metabolites at an initial rate that was 1.8-fold more rapid than that of the susceptible plants (Figure 2C). After 10 hours, the rates of accumulation were similar for both biotypes. The kinetics of accumulation of label in these products indicated that they were end-products of metabolic degradation. A number of conjugated products were produced by both biotypes. The compounds, although similar, were not identical. To date, technical difficulties have precluded a precise identification of their chemical structures. The physiological significance of this observation is therefore uncertain. At this stage we consider it unlikely that the small differences we have observed in metabolite levels are responsible for the thirty-fold difference in susceptibility to diclofop-methyl that is observed at the whole plant level. However, it must be stressed that small differences in metabolite pool sizes can result in large differences in internal concentrations in restricted intracellular compartments.

The mechanism of resistance to diclofop-methyl: a working model For reasons outlined both here and in two other reports in this volume (4, 9) we propose, as a working model, that the mechanism of resistance to diclofop-methyl and possibly to other aryloxyphenoxypropionate and cyclohexanedione graminicides in *L. rigidum* population SR31 is that shown in Figure 3. Two types of stress, both concentration- and time-dependent, are imposed upon the plants by diclofop. ACCase is inhibited and plasmamembranes are depolarised. As ACCase from both biotypes is equally inhibited by the graminicides we must assume that if the resistant plants survive then the concentrations of toxic chemicals in the immediate vicinity of the target enzyme must be reduced. We have as yet no firm evidence to suggest biotypic differences in the intracellular concentration of diclofop acid but have evidence that the resistant biotype can detoxify the herbicide at a slightly greater rate than the susceptible. It should be noted that the inhibition of ACCase is reversible and so inhibition will not necessarily be fatal unless the products of the reaction catalysed by the enzyme are immediately required for some vital process.

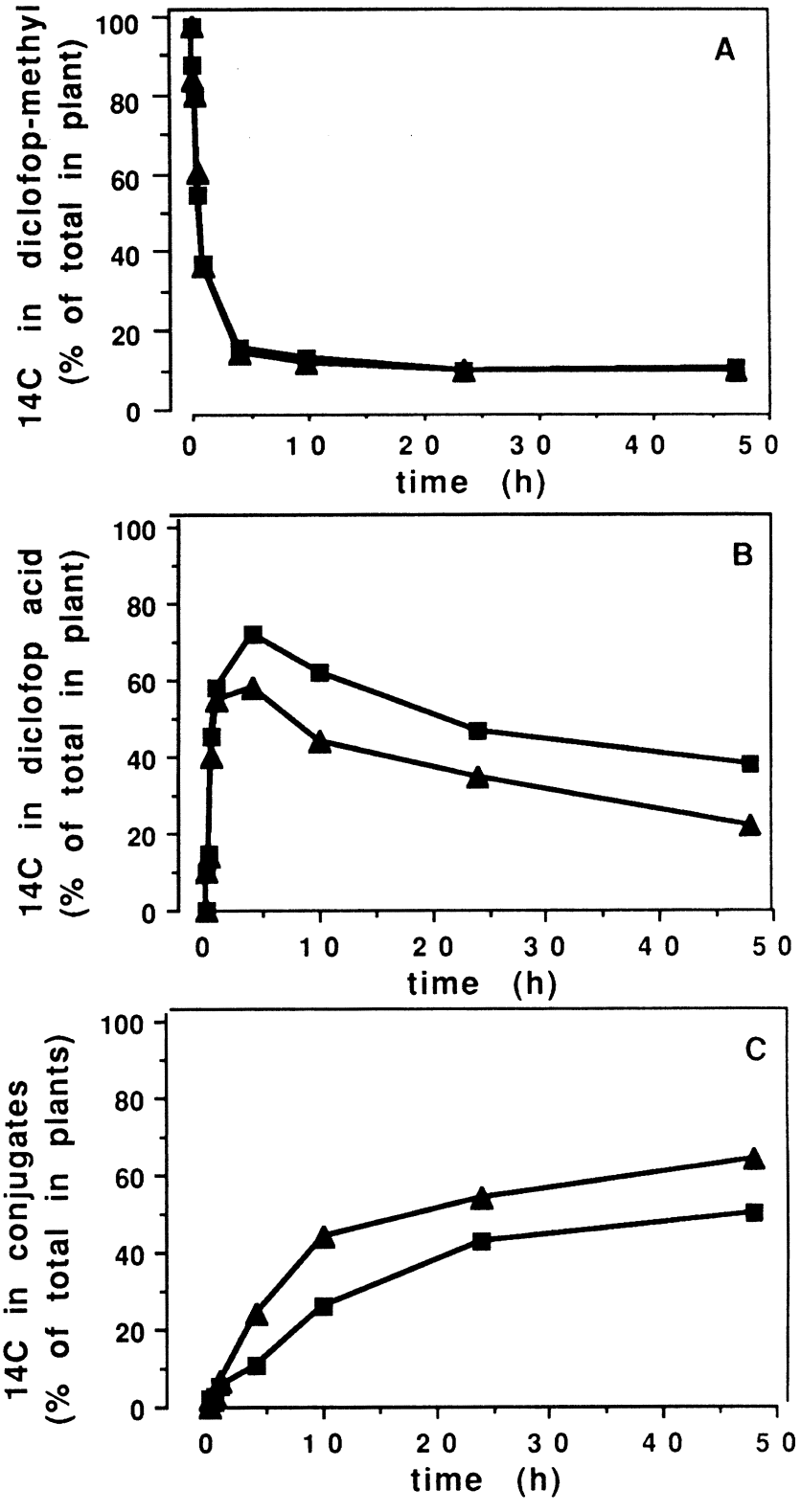

Figure 2. Radioactivity in (A) diclofop-methyl, (B) diclofop acid, and (C) diclofop-conjugates following exposure of susceptible (■) and resistant (▲) *L. rigidum* to ^{14}C-U-diclofop-methyl.

Plants were labelled by placing a 1 µl drop of 4.5 mM diclofop-methyl containing 35,000 dpm ^{14}C, in the leaf axil of each two-leaved plant. Rates of ^{14}C-diclofop-methyl uptake were similar in both biotypes with 70 % uptake occurring within 30 min.

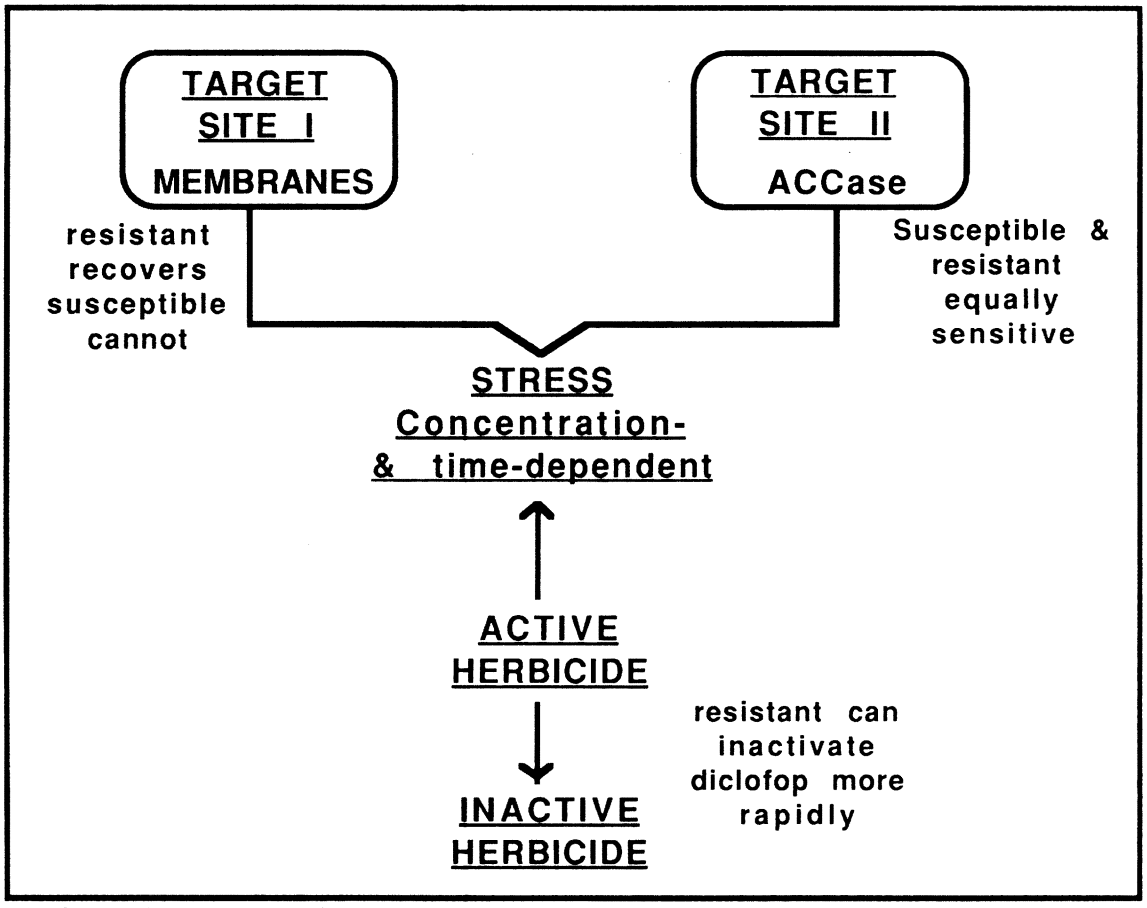

Figure 3. Model proposed for the mechanism of resistance of *L. rigidum* population SR31 to the aryloxyphenoxypropionate and cyclohexanedione herbicides. See also refs 4 and 9 this volume.

The aryloxyphenoxypropionate and cyclohexanedione graminicides can also depolarise membranes (4, 9). Only the resistant biotype can recover from this depolarisation. Depolarisation of transmembrane proton gradients interferes with a number of vital plant transport and growth processes. We suggest that, in biotype SR31, resistance to aryloxyphenoxypropionate and cyclohexanedione graminicides is principally the result of the ability of the biotype to repolarise membranes before irreversible damage occurs. The biochemical mechanism responsible for this repolarisation is not known.

ACKNOWLEDGEMENTS

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